Absorption / Transmission / Reflection Spectroscopy

An introduction to Absorption / Transmission / Reflection Spectroscopy

Ultraviolet Visible Near-Infra red (UV-Vis-NIR) spectroscopy is useful to characterize the absorption, transmission, and reflectivity of a variety of technologically important materials, such as pigments, coatings, windows, and filters. This more qualitative application usually requires recording at least a portion of the spectrum for characterization of the optical or electronic properties of materials.

Absorbance spectroscopy, commonly referred to as spectrophotometry, is the analytical technique based on measuring the amount of light absorbed by a sample at a given wavelength. Spectrophotometry, particularly in the visible and UV portions of the electromagnetic spectrum, is one of the most versatile and widely used techniques in chemistry and the life sciences.Molecular absorption spectroscopy in the ultraviolet (UV) and visible (VIS) is concerned with the measured absorption of radiation in its passage through a gas, a liquid or a solid. The wavelength region generally used is from 190 to about 1000nm, and the absorbing medium is at room temperature. However, in some cases (e.g. in enzyme assays)measurements at temperatures above or below room temperature may be advantageous or necessary.A molecule, or part of a molecule, that can be excited by absorption is called a chromophore. Organic chromophores which absorb strongly in the UV or visible portions of the spectrum nearly always involve multiple bonds such as C=C, C=O or C=N.This molecular excitation energy is usually dissipated as heat (kinetic energy) by the collision of the excited molecule with another molecule (e.g., a solvent molecule) as the molecule returns to the ground state. In other cases, the excitation energy is dissipated by the emission of light in a process called "fluorescence". In both cases, the intensity of the light transmitted by a collection of chromophores is less than the intensity of the incident light. An excited molecule can possess any one of a set of discrete amounts (quanta) of energy described by the laws of quantum mechanics. These amounts are called the "energy levels" of the molecule. In UV/VIS spectrophotometry, the major energy levels are determined primarily by the possible spatial distributions of the electrons and are called electronic energy levels, and to a lesser extent by vibrational energy levels, which arise from the various modes of vibration of the molecule (e.g. the stretching and bending of various covalent bonds).

The energy and wavelength of absorbtion is defined by the difference between energy levels of an electronic transition. This can be expressed by the following (equation 1):

$= hc/(E_2 - E_1)$

where:E_1 is the energy level of the molecule before absorption E_2 is an energy level reached by absorption

If all transitions were between only the lowest vibrational levels of the ground state and the first excited state, then an absorption spectrum would consist of narrow, discrete peak. However, the transition from one electronic level to the next level can occur between many vibrational levels also.

Since energy differences between vibrational levels within an electronic level are small compared to the minimum energy difference between electronic levels, the electronic transition consists of a cluster of very closely spaced spectral peaks. Each peak has significant width, comparable to the spacing between the peaks. This has the effect that the peaks overlap so much that a single broad peak, called an electronic absorption band, results.

For most molecules, absorption wavelengths corresponding to transitions between the ground state and any vibrational level of the first excited state, fall in the range of ultraviolet and visible light. Low-energy transitions are also possible between vibrational levels within a single electronic level. These transitions produce radiation in the infrared range. The figure on the right illustrates the relationship between specific energy transitions in the energy level diagram (upper graph) and the absorbance spectrum (lower graph).

Molecules which absorb photons of energy corresponding to wavelengths in the range 190 mm to about 1000nm exhibit UV/VIS absorption spectra. The quantized internal energy Eint of a molecule in its electronic ground or excited state can be approximated, with sufficient accuracy for analytical purposes, by the following (equation 2):

$$E_{int} = E_{el} + E_{vib} + E_{rot}$$

where: Eel is the electronic energyEvib the vibrational energyErot

the rotational energyAbsorption of a photon results in a change of the electronic energy accompanied by changes in the vibrational and rotational energies. Each vibronic transition, i.e. a particular electronic plus vibrational transition, corresponds to an absorption band consisting of rotational lines. In liquids and

Transitions to excited state

solids the rotational lines are broad and overlap so that no rotational structure is distinguishable.

The Beer-Lambert-Bouguer law, generally called the Beer-Lambert law, may be written for a single absorber either gaseous or in solution, i.e. as here in equation (3):

$$A = -\log_{10}(I_T/I_o) = -dc$$

where:I_T is the monochromatic radiant power transmitted by the absorbing mediumIo is the monochromatic radiant power incident on the medium $_i$ is the internal transmittance (=I_T/I_o) is the molar (decadic) absorption coefficient c is the amount concentration d is the absorption path length A is the (decadic) absorbance

Internal transmittance i, i.e. transmittance of the medium itself disregarding boundary effects, has to be distinguished from the

Beer-Lambert example

total transmittance . The difference, which is mainly due to reflection losses associated with cell windows, can be compensated by using matched cell. Absorptance (or absorption factor) is defined by = 1 - where reflection is assumed to be negligible.The Beer-Lambert law holds only if the absorbing species behave independently of each other and if the absorption occurs in a uniform medium.

Absorbance measurements allow the following: Determination of the concentration of a substance Kinetic assay of certain chemical reactions The identification of materials

The most common use of absorbance measurements is to determine the concentration of a solute. This can be done if the absorption coefficient () is known and Beer's law is obeyed.In practice, we do not generally rely on published values of because this quantity may be very sensitive to idiosyncrasies of reagent preparation and instrument design. Instead, it is normal practice to derive concentrations of unknown samples from a standard curve. local conditions of use std curve. The progress of a chemical reaction can be followed using a
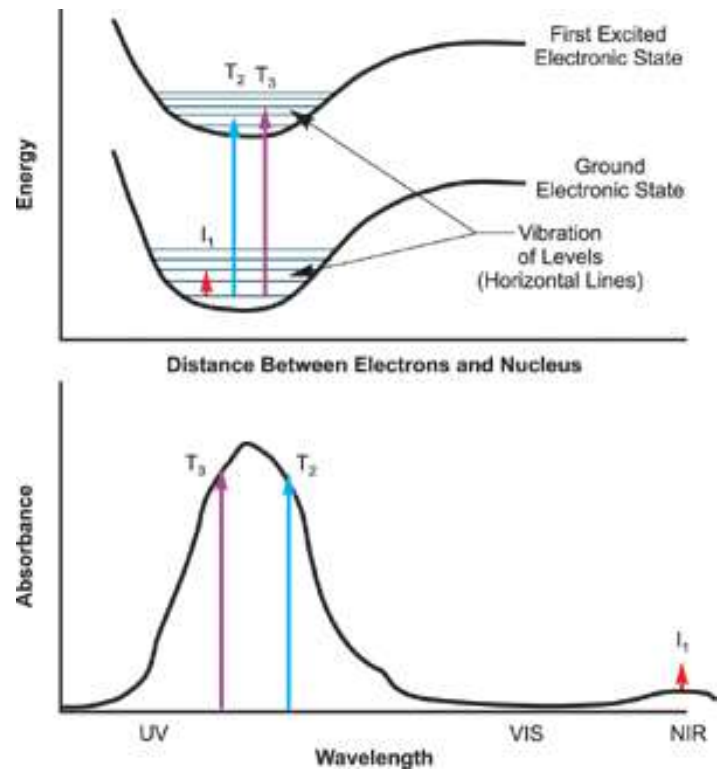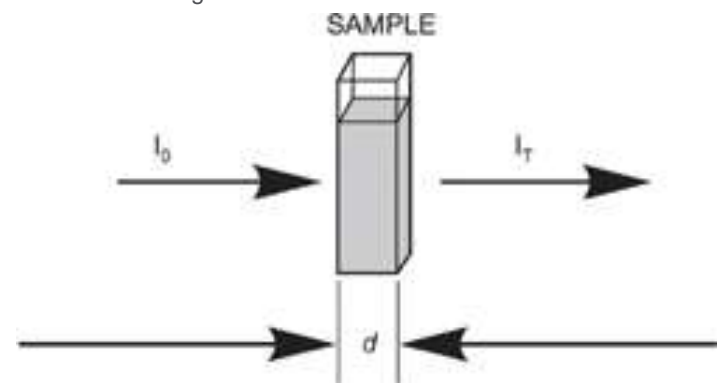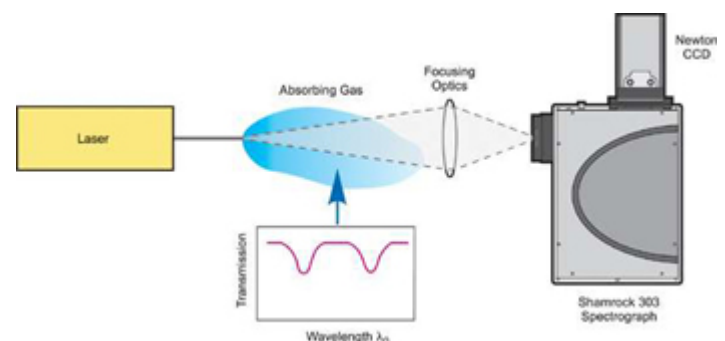

Illustration of Absorption Spectroscopy set-up

spectrophotometer to measure the concentration of either a reactant or a product over time. This forms the basis for the kinetic assays for many enzymes.Finally, most substances have characteristic absorbance spectra and can be identified thereby. This can be done by determining the complete spectrum and comparing it to spectra published in the literature or to the spectra of known reference materials.

Transmission spectroscopy is highly interrelated to Absorption Spectroscopy. This technique can be used for solid, liquid, and gas sampling. Here, light is passed through the sample and compared to light that has not. The resulting spectrum depends on the pathlength or sample thickness, the absorption coefficient of the sample, the reflectivity of the sample, the angle of incidence, the polarization of the incident radiation, and, for particulate matter, on particle size and orientation. In the Beer Lambert Law (equation 3) the term IT/Io is called transmittance. This form of spectroscopy has a setup similar to the one used for Absorption.

Reflectance spectroscopy is the study of light as a function of wavelength that has been reflected or scattered from a solid, liquid, or gas. As photons enter a mineral, some are reflected from grain surfaces, some pass through the grain, and some are absorbed. Those photons that are reflected from grain surfaces or refracted through a particle are said to be scattered. Scattered photons may encounter another grain or be scattered away from the surface so they may be detected and measured.

All materials have a complex index of refraction (equation 4):

$$m = n - jK$$

where:m is the complex index of refraction n is the real part of the index $j = (-1)1/2$ K is the imaginary part of the index of refraction, sometimes called extinction coefficient.

When photons enter an absorbing medium, they are absorbed according to the Beer-Lambert Law. The absorption coefficient is related to the complex index of refraction by the following (equation 5):

$$= 4K/$$

The reflection of light, R, normally incident onto a plane surface is described by the Fresnel equation (equation 6):

$$R = [(n - 1)^2 + K^2] / [(n + 1)^2 + K^2]$$

At angles other than normal, the reflectance is a complex trigonometric function involving the polarization direction of the incident beam.

Scattering can also be thought of as scrambling information. The information is made more complex, and because scattering is a non-linear process, recovery of quantitative information is difficult. Consider the simple Beers Law in equation 3. In transmission, light passes through a slab of material. There is little or no scattering (none in the ideal case; but there are always internal reflections from the surfaces of the medium). In reflectance, however, the optical path of photons is a random walk. At each grain the photons encounter, a certain percentage are absorbed. If the grain is bright, like a quartz grain at visible wavelengths, most photons are scattered and the random walk process can go on for hundreds of encounters. If the grains are dark, the majority of photons will be absorbed at each encounter and essentially all photons will be absorbed in only a few encounters. This process also enhances weak features not normally seen in transmittance, further increasing reflectance spectroscopy as a diagnostic tool.In a mixture of light and dark grains (e.g. quartz and magnetite) the photons have such a high probability of encountering a dark grain that a few percent of dark grains can drastically reduce the reflectance, much more than their weight fraction. A general rule with mixtures is that at any given wavelength, the darker component will tend to dominate the reflectance. The amount of light scattered and absorbed by a grain is dependent on grain size. A larger grain has a larger internal path where photons may be absorbed according to Beers Law. It is the reflection from the surfaces and internal imperfections that influence the scattering. In a smaller grain there are proportionally more surface reflections compared to internal photon path length, or in other words, the surface-to-volume ratio is a function of grain size. Absorptions in a spectrum have two components: continuum and individual features. The continuum is the "background absorption" onto which other absorption features are superimposed. It may be due to the wing of a larger absorption feature.

(1) External Reflection Spectroscopy. In this technique, also known as specular reflectance, light is reflected from a smooth (mirror-like) sample to record its spectrum. External reflectance is a non-destructive, non-contact technique. It is particularly useful for film thickness and refractive index measurements, as well as recording spectra of thin films on metal substrates. External reflection spectra may look different from transmission spectra in many ways, e.g. bands may be shifted to higher wavenumbers, spectra may follow the dispersion in the refractive index, and spectral contrast may not depend linearly on sample thickness.

(2) Internal Reflection Spectroscopy (IRS): for IRS, light is introduced into a suitable prism at an angle exceeding the critical angle for internal reflection. This produces an evanescent wave at the reflecting surface. A sample is brought in contact with or in close proximity to the reflecting surface. From the interaction of the evanescent wave with the sample, a spectrum can be recorded with little or no sample preparation. The IRS technique is non-destructive and can be used for solids, liquids, and powders.For thin films, the IRS spectra are the same as transmission spectra. For thick films, the absorption bands are more intense at longer wavelengths. As the angle of incidence approaches the critical angle, the bands tend to broaden on the long

wavelength side and the minima are displaced to longer wavelengths (lower wavenumbers). Dispersion type spectra are observed very close to and below the critical angle.

(3) Diffuse Reflection Spectroscopy: Spectra of powders and rough surfaces can be recorded by illuminating these surfaces and, with the aid of the appropriate optics, collecting sufficient scattered light for spectral analysis. This has been done routinely in the UV-VIS with integrating spheres and in the infrared with ellipsoids and paraboloids to collect the scattered radiation. Reaction chambers for diffuse reflectance allow the study of catalysis and oxidation reactions in situ. Since diffuse reflection spectra are the result of the interaction of light with the sample in every conceivable way, the spectra may exhibit features of transmission, external reflection and/or internal reflection.